REMARKS

I. PRELIMINARY REMARKS

Claims 1, 9-11, 18, 25, 32, 36-43, 51-53, 60, 67, 74, 78-85 and 93-106 are pending in the application. Claims 11, 18, 32, 39 and 42 have been amended to correct minor errors for clarity. Claims 43, 51-53, 60, 67, 74, 78-85, 93 -104 and 106 have been canceled to simplify the issues to place the case in condition for allowance. No new matter has been introduced by the amendments.

The present invention is directed to the discovery that administration of hydroxycitric acid (HCA) is effective to decrease ghrelin levels in a person or other mammal in need thereof and provides methods for administering HCA to subjects for the reduction of ghrelin.

II. THE OUTSTANDING REJECTIONS

Claims 1, 9-11, 18, 25, 43, 51-53, 60, 67, 85 and 93-97 stand rejected under 35 U.S.C. §102(e) as being anticipated by Bhaskaran et al., U.S. Publication No. 2003/0207942 ("Bhaskaran").

Claims 1, 9, 10, 43, 51, 52, 85, 93, 94, 105, and 106 stand rejected under 35 U.S.C. §102(b) as being anticipated by Balasubramanyam et al., U.S. Patent No. 6,160,172 ("Balasubramanyam").

Claims 1, 9-11, 18, 25, 32, 36-43, 51-53, 60, 67, 74, 78-85 and 93-106 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Raju, International Publication No. WO99/03464 ("Raju") in view of one or more of Policappelli, U.S. Patent No. 5,612,039 ("Policappelli"); Allen, U.S. Patent No. 5,480,657 ("Allen"); Alviar et al., U.S. Patent No. 6,413,545 ("Alviar"); and Balasubramanyam.

Claims 85 and 93-104 stand provisionally rejected for nonstatutory obviousness-type double patenting over claims 25-27, 29, and 31-41 of copending Application No. 09/463,024.

III. PATENTABILITY ARGUMENTS

A. The Rejections over Bhaskaran and Balasubramanyam under 35 U.S.C. §§ 102(e) and 102(b) Should be Withdrawn.

The anticipation rejections over Bhaskaran and Balasubramanyam should be withdrawn because decreased ghrelin levels cannot be considered inherent in all compounds that are associated with weight reduction. Indeed, the references attached and discussed below show that obesity is associated with high *and* low levels of ghrelin. Therefore, decreased ghrelin levels cannot be considered inherent in all compounds that are associated with weight reduction. Thus, while both references teach administration of HCA to overweight subjects, 1) most overweight subjects have low ghrelin levels (and would not appear to be in any need of reducing their ghrelin levels) and 2) many subjects with high ghrelin levels are not overweight. There is nothing in the prior art that discloses that decreased ghrelin levels are necessarily associated with weight loss.

First, it is important to point out that neither Bhaskaran nor Balasubramanyam nor the other cited references disclose any effect of HCA on ghrelin levels. Applicants have demonstrated that HCA alone has substantial ghrelin reducing effects (See Figure 1 which reports that administration of HCA decreases serum ghrelin levels by 20.2%)

1. The Application Claims a "New Use" not an "Added Benefit."

Nevertheless, the outstanding rejection appears to be based on the argument that prior uses of HCA as an appetite suppressant inherently anticipate the present claims. Such is not the case because the present claims do not represent a mere added benefit of administering HCA to suppress appetite, but rather claim a new use of the compound to treat patients in need of decreased ghrelin levels.

There is a distinction between a new use and an added benefit. A new use for an existing compound may be the subject of a valid patent. Indeed there is express statutory authority allowing a patent on a process which is a new use of a known process, composition of matter, or material provided that the new use is unobvious and not subject to a statutory one year time bar, neither of which apply in this case.

On the other hand, an unpatentable added benefit may be viewed as "a newly discovered result [] of [a] known process [] directed to the same purpose." *Bristol-Myers Squibb Co. v. Ben Venue Labs. Inc.* 246 F. 3d 1368, 1376 (Fed. Cir. 2001).

In *Rapoport v. Dement* 254 F.3d 1053, 1063 (Fed Cir 2001) the Federal Circuit found that a patent claiming a method of using a known compound to treat sleep apnea was not inherently anticipated by a prior art reference disclosing the use of the same compound in the treatment of anxiety. The court made this determination in spite of the fact that anxiety was a known symptom of sleep apnea. Thus, although the drug was the same, the implications of its administration for two different purposes precluded a finding of inherent anticipation.

The present claims do not represent a mere added benefit of administering HCA to suppress appetite, but rather claim a new use of the compound to treat patients in need of decreased ghrelin levels. Such patients might not have a need for appetite suppression and would not have been treated by the prior art administration of HCA. Instead, such patients might be in need of decreased ghrelin levels to regulate secretion of pituitary growth hormone (GH), as well as GHS-Rs distributed in hypothalamic neurons and in the brainstem. Ghrelin also appears to be involved in mesolimbic cholinergic-dopaminergic reward link, a circuit that communicates the hedonic and reinforcing aspects of natural rewards, such as food, as well as of addictive drugs, such as ethanol. In discovering the utility of HCA administration to subjects in need of ghrelin reduction Applicants have disclosed something to the public that it did not previously possess. It is the role of the patent system to reward such advancement in the arts.

2. Most (but not all) Overweight Subjects Have Low Ghrelin Levels and it is Unexpected that HCA Administration Would Lower Ghrelin Levels.

In general, overweight individuals are surprisingly characterized by low ghrelin levels compared to normal weight individuals and underweight individuals, such as those suffering from anorexia nervosa, who have high plasma ghrelin levels. Ghrelin, http://arbl.cvmbs.colostate.edu/hboods/pathphys/endocrin/gi/ghrelin.html (Attached hereto as Exhibit A). Thus, to the extent that Bhaskaran and Balasubramanyam teach administration of HCA to overweight subjects, it would not be expected that HCA would lower ghrelin levels.

Moreover, not all (or even most) overweight subjects have high ghrelin levels and it is not clear from the prior art that all overweight subjects need their ghrelin levels reduced. While many overweight subjects have low ghrelin levels (e.g., subjects suffering from nonalcoholic fatty liver disease tend to be overweight but have low ghrelin levels. *See* Marchesini et al., J. Clinical Endocrinology and Metabolism, vol. 88, No. 12, 5674-5679 (2003) (Attached hereto as Exhibit B)), others such as those suffering from Prader-Willi syndrome have plasma ghrelin levels that are "exceptionally high in comparison to patients similarly obese." (Exhibit A). The Action states that ghrelin is a "target for development of anti-obesity treatments." Action at p.12. However, there is nothing in the prior art disclosing that administration of HCA would lower ghrelin levels.

It was therefore unclear, prior to the disclosure of the present application, whether administration of HCA to overweight subjects would have had any affect on ghrelin levels. Moreover, there is no evidence that administration of HCA to overweight subjects in the prior art might not have invariably decreased ghrelin levels because the ghrelin levels might not be high and such invariability is necessary for inherent anticipation. Nevertheless, and in it absence, the rejections under 35 U.S.C. §102 must be withdrawn.

3. Appetite Suppressors Do Not Invariably Reduce Ghrelin Levels.

The outstanding inherent anticipation rejection is improperly made on the grounds that the references teach administering HCA to reduce body weight and that "the functionality (i.e., decreasing ghrelin levels) would also be the same." (Action at page 3, lines 8-9).

This basis for anticipation should be withdrawn because neither Bhaskaran nor Balasubramanyam associates decreased ghrelin levels with hydroxycitric acid or gymnemic acid and suppressing appetite does not inherently decrease ghrelin levels.

In the absence of some disclosure that HCA decreases ghrelin levels, an inherent anticipation rejection on the basis that appetite suppression/weight loss agents inherently decrease ghrelin levels must demonstrate that that proposition is invariably so. While some weight control agents manipulate ghrelin levels, most do not and it is not invariable that weight control agents decrease ghrelin levels. For example, Briggs et al., U.S. Patent Publication No. 2004/0204472 discloses numerous weight loss agents in 59 chemical classes only one of which relates to ghrelin. Briggs, paragraph [1285].

> "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993)...Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999)" MPEP 2112 IV, 2100-47 (Rev. 5, Aug. 2006)

Accordingly, it is improper to reject the claims directed to decreasing ghrelin levels in a person or other mammal on the basis that some but by no means all or even many weight management compositions decrease ghrelin levels. Applicants have discovered (and there is no dispute that they were the first to recognize) that such is the case for HCA but reliance on the applicants' disclosure to show that is improper hindsight. For these reasons, the anticipation rejection of claims 1, 9-11, 18, and 25 over Bhaskaran et al. and Balasubramanyam et al. should be withdrawn.

B. The Rejection under 35 U.S.C. §103(a) Should Be Withdrawn Because There Is No Teaching in the Art that Administration of HCA Would Reduce Ghrelin Levels.

The rejection under 35 U.S.C. §103(a) over Raju in view of one or more of Policappelli, Allen, Alviar, and Balasubramanyam should be withdrawn because the claims are directed to methods for decreasing ghrelin levels in subjects in need thereof by administering sufficient amounts of hydroxycitric acid (HCA) and there is no teaching in the art that administration of HCA would reduce ghrelin levels.

Each of Raju, Policappelli, Alviar, and Balasubramanyam teach that HCA promotes weight loss and Allen teaches that niacin-bound chromium promotes weight loss but none of these references teach that HCA (or chromium for that matter) decrease ghrelin levels.

There are many biological pathways for weight loss agents, and prior to the disclosure of the present application, it could not be predicted under which pathway HCA would operate. Biological activities relating to weight loss include (1) catecholamine modulators, (2) norepinephrine, dopamine and serotonin reuptake inhibitors, (3) lipase inhibitors, (4) dual serotonin reuptake inhibitors and serotonin releasing agents, (5) aminoketone class antidepressants, (6) activators of ATP-dependent K^+ channels, (7) anti-

hyperglycemics, (8) GABA enhancer and sodium channel blockers, (9) serotonin and dopamine releasers, (10) histamine-3 antagonists, (11) cannabinoid (CB1) receptor antagonists, (12) alpha adrenergic receptor agonists, (13) melanocortin-4 receptor agonists, (14) neuropeptide Y antagonists, (14) beta(3)-adrenergic agonists, (15) glucagon-like peptide-1 agonists, (16) PPAR-gamma antagonists and PPAR-gamma partial antagonists, (17) urocortin agonists, (18) CCK agonists, (19) UCP activating agents, (20) prolactin modulators, (21) growth-hormone secratagogues, (22) ciliary neurotropic factors, (23) antihistamines, (23) 5-HT_{2C} agonists, (24) 5-HT_{2A} agonists, (25) dopamine agonists, (26) adipocyte complement-related protein (Acrp30) modulators, (27) cannabinoid antagonists, (28) tyrosine phosphatase modulators, (29) 11beta hydroxysteroid dehydroxysteroid dehydrogenase type 1 modulators, (30) cyclic AMO response element-binding protein modulators, (31) diacylglycerol o-acyltransferase modulators, (32) fatty acid transport protein 4 modulators, (33) G protein beta-3 subunit 825T modulators, (34) high mobility group 1C modulators, (35) Kallikrein modulators, (36) melanin-concentrating hormone receptor modulators, (37) perilipin modulators, (38) Tub gene modulators, (39) anticonvulsants, (40) leptin receptor modulators, (41) metabolic accelerators, (42) adipogenesid modulating agents, (43) HK-a receptor antagonists, (44) PPAR-gamma antagonists, (45) PPAR-alpha agonists and (46) leptin agonists. Briggs et al., US 2004/0204472.

There is no evidence that decreased ghrelin activity would have been predicted for HCA from an examination of the art prior to Applicants' invention. Accordingly, the rejection should be withdrawn.

C. The Rejection for Obviousness-Type Double Patenting Should be Withdrawn.

The provisional rejection of claims 85 and 93-104 for obviousness-type double patenting over copending Application No. 09/463,024 should be withdrawn in light of the cancellation of these claims.

IV. CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance. Should the Examiner wish to discuss any issues of form or substance in order to expedite allowance of the pending application, he is invited to contact the undersigned at the number indicated below.

Dated: May 20, 2009

Respectfully submitted,

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Inventors: Debasis Bagchi et al.
Title: "Method and Composition for Decreasing Ghrelin Levels"
U.S. Serial No.: 10/805,129
Atty. Docket No.. 31174/30019

EXHIBIT "A"

Endocrine Index Glossary

Ghrelin

Structure of Ghrelin and Its Receptor

Ghrelin is synthesized as a preprohormone, then proteolytically processed to yield a 28-amino acid peptide. An interesting and unique modification is imposed on the hormone during synthesis in the form of an n-octanoic acid bound to one of its amino acids; this modification is necessary for biologic activity.

Synthesis of ghrelin occurs predominantly in epithelial cells lining the fundus of the stomach, with smaller amounts produced in the placenta, kidney, pituitary and hypothalamus.

The ghrelin receptor was known well before ghrelin was discovered. Cells within the anterior pituitary bear a receptor that, when activated, potently stimulates secretion of growth hormone - that receptor was named the **growth hormone secretagoue receptor** (GHS-R). The natural ligand for the GHS-R was announced in 1999 as ghrelin, and ghrelin was named for its ability to provoke growth hormone secretion (the suffix ghre means "grow").

Ghrelin receptors are present on the cells in the pituitary that secrete growth hormone, and also have been identified in the hypothalamus, heart and adipose tissue.

Control and Physiologic Effects of Ghrelin

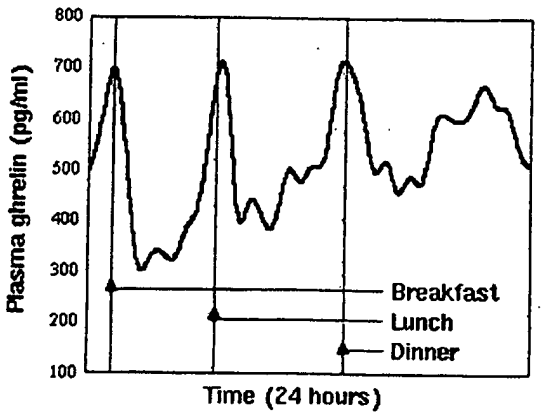
At least two major biologic activites have been ascribed to ghrelin:

- **Stimulation of growth hormone secretion:** Ghrelin, as the ligand for the growth hormone secretagogue receptor, potently stimulates secretion of growth hormone. The ghrelin signal is integrated with that of growth hormone releasing hormone and somatostatin to control the timing and magnitude of growth hormone secretion.
- **Regulation of energy balance:** In both rodents and humans, **ghrelin functions to increase hunger** though its action on hypothalamic feeding centers. This makes sense relative to increasing plasma ghrelin concentrations observed during fasting (see below). Additionally, humans injected with ghrelin reported sensations of intense hunger. Ghrelin also appears to **suppress fat utilization in adipose tissue**, which is somewhat paradoxical considering that growth hormone has the opposite effect. Overall, ghrelin seems to be one of several hormonal signals that communicates the state of energy balance in the body to the brain.

Other effects of ghrelin include stimulating gastric emptying and having a variety of positive effects on cardiovascular function (e.g. increased cardiac output). It is not totally clear whether the cardiovascular effects are a direct effect of ghrelin or represent an indirect effect of ghrelin's ability to stimulate growth hormone secretion.

Blood concentrations of ghrelin are lowest shortly after consumption of a meal, then rise during the fast just prior to the next meal. The figure to

the right shows this pattern based on assays of plasma ghrelin in 10 humans during the course of a day.



Adapted from Cummings et al. Diabetes 50:1714, 2001.

Given the effects of ghrelin on energy metabolism and hunger, it is a prominent target for development of anti-obesity treatments. It has been reported that immunization of rats against ghrelin resulted in decreased weight gain and adiposity relative control rats, even though both groups consumed an equivalent amount of food. This intriguing experiment suggests the possibility of a vaccine against obesity.

Disease States

Ghrelin concentrations in blood are reduced in obese humans compared to lean control subjects, but whether this is cause or effect is not defined. Patients with anorexia nervosa have higher than normal plasma ghrelin levels, which decrease if weight gain occurs.

Prader-Willi syndrome is another disorder relevant to ghrelin science. Affected patients develop extreme obesity associated with uncontrollable and voracious appetite. The plasma ghrelin levels are exceptionally high in comparison to patients similarly obese due to other causes. Prader-Willi syndrome is clearly a complex disease with many defects; it may be that excessive ghrelin production contributes to the appetite and obesity components.

Index of: Gastrointestinal Hormones

◀ **Secretin** **Motilin** ▶

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EXHIBIT B

EXHIBIT "B"

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Low Ghrelin Concentrations in Nonalcoholic Fatty Liver Disease Are Related to Insulin Resistance

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Abstract

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Several physiological and pathophysiological conditions, including changes in body fat, food intake, and insulin resistance, are known to be associated with variations in plasma ghrelin concentrations. We tested the hypothesis that insulin resistance exerts a primary role by measuring ghrelin in 86 patients with nonalcoholic fatty liver disease (NAFLD), a condition in which insulin resistance is relatively independent of obesity. Compared with 40 matched healthy subjects, patients with NAFLD had similar glucose levels and higher plasma insulin and insulin resistance [homeostasis model assessment (HOMA)-R index] by over 60%. Ghrelin was reduced (mean ± SD, 226 ± 72 pmol/liter in NAFLD *vs.* 303 ± 123 in controls; $P < 0.0001$). In relation to quartiles of body mass index, ghrelin progressively decreased in

controls ($P = 0.003$), but not in patients ($P = 0.926$). In relation to quartiles of HOMA-R, ghrelin decreased in both groups, and significantly correlated with HOMA-R. After adjustment for age and sex, HOMA-R was the sole factor significantly associated with low ghrelin in the whole group (odds ratio, 5.79; 95% confidence interval, 2.62–12.81; $P < 0.0001$) and specifically in NAFLD (2.96; 1.12–7.79; $P = 0.028$). The study suggests that insulin resistance is a major factor controlling ghrelin levels in subjects with and without NAFLD.

Introduction

GHRELIN IS A novel peptide involved in food intake and energy balance. Animal models have shown that ghrelin promotes energy sparing, finally leading to increased body weight. When injected in both humans and animals, ghrelin stimulates hunger (1), thus increasing food intake. In relation to these orexigenic properties, ghrelin has been extensively investigated as a possible cause of obesity. Surprisingly, the circulating levels of ghrelin are low in obese subjects when compared with normal controls (2), and increase sharply after weight loss (3). By contrast, ghrelin levels are elevated in constitutionally thin subjects with low body mass index (BMI) (4). The relative roles of either total body fat or associated metabolic conditions in regulating ghrelin concentrations have never been clarified. Increased body fat is generally associated with hyperinsulinemia and insulin resistance, and a correlation was reported between insulin levels or quantitative measures of insulin resistance and ghrelin concentrations in normal (5) and pathological conditions (6).

Nonalcoholic fatty liver disease (NAFLD) is a complex metabolic condition in which both lifestyle and genetic factors have a pathogenic role (7). It has been convincingly associated with the metabolic insulin resistance syndrome; most patients are overweight or frankly obese, with altered glucose regulation, dyslipidemia, and raised blood pressure, all contributing to the disorder (8). However, large studies have shown that approximately 10–20% of patients are lean and have normal glucose regulation, but are nonetheless insulin resistant when tested by the homeostasis model assessment (HOMA) method (9) or by the euglycemic clamp technique (10).

In the present study, we measured fasting ghrelin concentration in a large series of NAFLD patients with different phenotypes to test the relative importance of body fat, glucose regulation, hyperinsulinemia, and insulin resistance in ghrelin levels.

Patients and Methods

Patients

Eighty-six NAFLD patients (79 males) (median age, 38 yr; range, 19–74 yr) and 40 control subjects (32 males) (median age, 43 yr; range, 28–77 yr) were included in the study. Their clinical and laboratory variables are presented in Tables 1 and 2. In NAFLD cases, the diagnosis was based on chronic hypertransaminasemia (alanine transaminases (ALT) of >1.5

times the upper normal values for 3 months or more), negative hepatitis B and C viral markers, absence of autoantibodies indicative of autoimmune hepatitis or celiac disease, negative or negligible alcohol consumption (<140 g/wk), and bright liver at ultrasound scanning. The diagnosis was confirmed by liver biopsy in 62 cases. According to the criteria proposed by Brunt *et al.* (11), 46 cases were classified as nonalcoholic steatohepatitis (NASH), and 16 were classified as pure fatty liver. Control subjects were free of hepatic and endocrine diseases. They were selected in a BMI range similar to that of NAFLD cases. Previously diagnosed diabetes mellitus [American Diabetes Association classification (12)] was an exclusion criterion for both NAFLD patients and control subjects. In 54 NAFLD patients and 28 controls, an oral glucose tolerance test (OGTT) was also performed for a complete evaluation of glucose tolerance.

View this table: [in this window] [in a new window] **TABLE 1.** Phenotypic data of NAFLD patients and control subjects

View this table: [in this window] [in a new window] **TABLE 2.** Biochemical and clinical data of NAFLD and control subjects (mean ± SD)

All patients were regularly followed as outpatients and were on a controlled dietary regimen, comprising 25 kcal/kg body weight per day at the time of study. Blood samples for ghrelin concentrations were collected between 0800 and 0900 h, after an overnight fast. Plasma was immediately separated and stored at -80 C until analysis.

The purpose of the study was explained to all subjects, who gave their informed consent to blood sampling for ghrelin measurement. All other investigations were carried out during regular follow-up of NAFLD patients, according to specific protocols. The study was approved by the senior staff committees of the two university hospitals, institutional review boards regulating noninterventional studies.

Methods

Anthropometry. Body weight was measured in light clothing and without shoes to the nearest half-kilogram. Height was measured to the nearest half-centimeter. BMI was calculated as weight (kilograms) divided by height squared (square meters). Subjects with BMI between 25 and 30 kg/m^2 and greater than or equal to 30 kg/m^2 were considered overweight and obese, respectively. Waist circumference was measured at the nearest half-centimeter at the shortest point below the lower rib margin and the iliac crest, whereas hip circumference was similarly obtained at the widest point between hip and buttock. Body fat distribution was also evaluated by waist-to-hip ratio (WHR) according to World Health Organization (13).

Biochemical and hormonal measurements. Plasma immunoreactive ghrelin levels were measured in duplicate using a commercially available RIA (Phoenix Pharmaceuticals, Inc.,

Mountain View, CA) that uses ^{125}I-labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody raised against the C-terminal part of human ghrelin (6). Intraassay and interassay coefficients of variation were less than 5.3 and 13.6%, respectively. This assay recognizes both acylated and deacylated ghrelin (2). The antiserum does not cross-react with any relevant peptide as previously shown (2, 14).

Plasma glucose was measured in duplicate with an automated analyzer. The coefficient of variation for any single determination was ±1.5%. Insulin was measured by an immunoenzymometric assay (AIA-PACK IRI, AIA-1200 system; Tosoh Co., Tokyo, Japan) with intraassay and interassay coefficients of variation for the quality control of less than 7%. Insulin resistance was calculated on the basis of fasting values of plasma glucose and insulin, according to the HOMA method (15) as follows: insulin resistance [HOMA-R (%)] = IRI_0*BG_0/22.5, where fasting insulin (IRI_0) is in microunits per milliliter, and glucose (BG_0) is in millimoles per liter.

In patients submitted to OGTT, two more indices of insulin sensitivity were derived from basal and postload glucose and insulin concentrations. The insulin sensitivity index (ISI) was calculated according to Matsuda and DeFronzo (16) as follows:

$$ISI = \frac{10,000}{\sqrt{(BG_0 * IRI_0) * (\text{average } BG_{0-120} * \text{average } IRI_{0-120})}},$$

whereas the sensitivity index (SI) proposed by Cederholm and Wibell (17) was calculated as follows:

$$SI = \frac{75,000 + (BG_0 - BG_{120}) * 0.19 * BW}{120 * \text{average } BG_{0-120} * \log(\text{average } IRI_{0-120})},$$

where average BG_{0-120} and average IRI_{0-120} represent the mean of individual values measured after glucose load. Body weight (BW) also enters the SI equation, with a correction factor for glucose space. Additional correction factors are needed to transform metric units into SI units.

Fasting serum cholesterol, high-density lipoprotein (HDL) cholesterol, uric acid, and triglyceride levels were measured by routine laboratory techniques.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Inc., Cary, NC.). Patients were grouped according to categorical variables (sex, class of BMI, presence/absence of impaired glucose regulation, and hypertension). Ghrelin concentrations were tested for significance using unpaired *t* test (two-tail) or nonparametric analysis (Mann-Whitney *U* test or Kruskal-Wallis test). Contingency test and Fisher's exact test were also used, whenever appropriate, to compare prevalence.

Logistic regression analysis was used to determine factors more closely associated with low ghrelin concentrations. For this purpose, a ghrelin concentration below the median of the whole

group (235 pmol/liter) was considered as dependent variable, and the clinical and laboratory values of Tables 1 and 2 were tested in univariate analysis. After this, variables significantly associated with low ghrelin were tested for independency in multivariate logistic regression analysis, after correction for age and sex. Two different models were built to further adjust data for BMI and waist circumference, separately.

All data in the text and in the tables are given as means ± SD, when not otherwise indicated. Values of $P < 0.05$ were considered statistically significant.

Results

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Anthropometric and clinical data

Both controls and NAFLD patients were selected in a wide BMI range (controls, 19.2–35.2 kg/m^2; NAFLD, 20.9–37.9 kg/m^2), without differences in the distribution among BMI classes between groups. However, a larger waist circumference and a higher WHR, indicative of visceral adiposity, characterized NAFLD patients.

At history, diabetes, hypertension, and dyslipidemia were not more prevalent in NAFLD, but at the time of study, arterial pressure was higher in NAFLD, whereas HDL-cholesterol concentrations were lower. NAFLD patients were also characterized by a different response to OGTT. In particular, the prevalence of impaired glucose tolerance was remarkably higher in NAFLD (22.2 *vs.* 3.6% in controls; $P = 0.030$, Fisher's exact test) without differences in the prevalence of OGTT-detected diabetes (9.3% in NAFLD *vs.* 7.1% in controls; $P = 0.745$).

Fasting glucose was not different, but both insulin concentrations and HOMA-R were increased by over 60%. The 95% confidence interval of HOMA-R in normal-weight controls ranged up to 2.74%. This cutoff was used as the upper limit of normal insulin sensitivity. According to this criterion, 13 controls were considered to be insulin resistant (32%) *vs.* 57 of 86 NAFLD cases (66%; $P = 0.0005$, Fisher's exact test). A significant correlation was observed between BMI and HOMA-R in controls ($r = 0.334$; $P = 0.035$), but not in NAFLD patients ($r = 0.202$; $P = 0.062$).

Also, the two indices of insulin sensitivity derived by OGTT (ISI and SI) showed a significant resistance to insulin activity in the course of the glucose load in the NAFLD cohort when compared with control subjects.

Ghrelin levels

Ghrelin levels were reduced in NAFLD patients (226 ± 72 *vs.* 303 ± 123 pmol/liter in controls; $P < 0.0001$). Differences were maintained when levels were analyzed in relation to gender (males, NAFLD, 224 ± 74 pmol/liter, controls, 279 ± 100 pmol/liter, $P = 0.002$; females, NAFLD, 247 ± 35 pmol/liter, controls, 396 ± 164 pmol/liter, $P = 0.035$). Importantly, a gender effect was observed only in the control group (males *vs.* females, $P = 0.014$), whereas no statistically significant difference between males and females was observed in NAFLD.

When ghrelin levels were analyzed in relation to quartiles of BMI, in control subjects, ghrelin progressively decreased from 401 ± 130 (lower quartile; BMI range, 19.2–25.9 kg/m^2) to 236 ± 75 (upper quartile; BMI range, 30.2–35.2 kg/m^2; $P = 0.003$). In NAFLD patients, ghrelin levels were similar between subjects in the lower BMI quartile (242 ± 90 pmol/liter; BMI range, 20.9–24.6 kg/m^2) compared with subjects in the upper quartile (244 ± 72 pmol/liter; $P = 0.926$; BMI range, 29.0–37.9 kg/m^2) (Fig. 1⊞). A significant correlation between BMI and ghrelin concentrations was present in controls (r = -0.604; $P < 0.0001$), but not in NAFLD (r = -0.093; $P = 0.397$) (Fig. 2⊞).

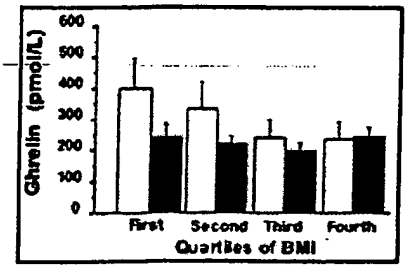

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FIG. 1. Ghrelin concentrations (mean ± 2 SE) in control subjects (□) and in NAFLD patients (■) in relation to quartiles of BMI. Significant differences are present in controls ($P = 0.0043$; Kruskal-Wallis test), but not in liver patients ($P = 0.083$).

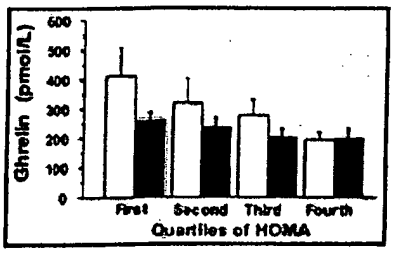

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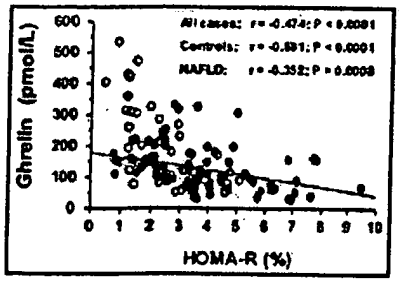
FIG. 2. Ghrelin concentrations (mean ± 2 SE) in control subjects (□) and in patients with NAFLD (■) in relation to quartiles of the HOMA-R index. Significant differences are present both in controls ($P = 0.0009$; Kruskal-Wallis test) and in liver patients ($P = 0.0014$).

In relation to quartiles of HOMA-R, a progressive decrease of average ghrelin concentrations was observed in both groups (Fig. 2⊞), and the correlations between HOMA-R and ghrelin were both statistically significant (controls, r = -0.591, $P < 0.0001$; NAFLD, r = -0.352, $P = 0.0008$), although the two regression lines were considerably different, both in the intercept and in the slope (Fig. 3⊞).

FIG. 3. Correlation between insulin resistance (HOMA-R) and ghrelin concentrations in control subjects (○, dotted line) and in patients with NAFLD (•, continuous line). The r coefficients of correlation and P values are separately reported for the whole group (n = 126), for control subjects (n = 40), and for



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fatty liver patients (n = 86).

Less significant correlations were observed between ghrelin levels and other indices of visceral adiposity (waist circumference and WHR), as well as with indices of insulin sensitivity derived from OGTT (ISI and SI). In NAFLD patients, ghrelin failed to correlate with liver function tests (albumin and prothrombin time), as well as with liver cell necrosis (ALT, r = 0.033, $P = 0.766$).

Factors associated with ghrelin concentrations were further tested by logistic regression analysis (Table 3⊞). Ghrelin concentrations below the median of 235 pmol/liter were associated with several laboratory and anthropometric indices at univariate analysis. The association of low ghrelin with both insulin and insulin resistance was maintained after adjustment for BMI, either in the whole population or in NAFLD patients (not reported in details).

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TABLE 3. Factors associated with low ghrelin concentrations (<235 pmol/liter) at univariate and multivariate analysis

After adjustment for age and sex, a HOMA-R indicative of insulin resistance (≥2.74%) was the sole factor associated with low ghrelin concentrations at multivariate analysis in the whole population (Table 3⊞), as well as separately in control subjects [odds ratio (OR), 18.90; 95% confidence interval, 3.52–101.48; $P = 0.0006$] and in NAFLD patients (OR, 2.96; 95% confidence interval, 1.12–7.79; $P = 0.028$). BMI did not enter the regression, and when data were adjusted for BMI, the effects of HOMA-R on ghrelin levels did not change (all cases, 5.58, 2.50–12.43, $P < 0.0001$; controls, 16.68, 2.80–99.28, $P = 0.002$; NAFLD, 2.83, 1.07–7.52, $P = 0.037$).

In the model in which data were further adjusted for waist circumference, in addition to age and gender, HOMA-R was similarly the sole factor significantly associated with low ghrelin, both in the whole population (OR, 6.00; 95% confidence interval, 2.42–12.96; $P < 0.0001$) and in NAFLD patients (2.92; 95% confidence interval, 1.08–7.85; $P = 0.034$).

NAFLD patients submitted to liver biopsy were not different from subjects who had not had a liver biopsy in the parameters presented in Table 2⊞. In particular, BMI was 27.3 ± 3.3 kg/m^2 in patients who had and 26.5 ± 2.8 kg/m^2 in patients who had not had a liver biopsy ($P = 0.375$),

and HOMA-R was 3.89 ± 1.83 and 3.68 ± 2.14%, respectively ($P = 0.673$). Also, ALT values were similar (biopsy positive, 84 ± 38 U/liter; biopsy negative, 73 ± 39 U/liter; $P = 0.294$). Ghrelin levels were also similar (234 ± 78 and 224 ± 71 pmol/liter; $P = 0.606$), and they did not differ in relation to the severity of fat deposition, fibrosis, and necroinflammatory activity. In particular, ghrelin was not different when patients were classified according to the presence/absence of NASH (pure fatty liver, 220 ± 88 pmol/liter; NASH, 226 ± 69 pmol/liter; $P = 0.781$).

Discussion

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Our data clearly show that ghrelin levels are reduced in NAFLD patients, after correction for sex, age, and BMI. Therefore, NAFLD may be included in the growing group of pathological conditions characterized by low ghrelin concentrations. The most likely reason for low fasting ghrelin is insulin resistance, which strictly correlates with ghrelin levels both when the two cohorts were examined together, and separately in NAFLD patients and in control subjects.

NAFLD is significantly associated with the metabolic syndrome (8). Although most patients are overweight or obese, 10–20% of cases have a BMI within normal limits. This condition represents a suitable model to study the relationship of insulin sensitivity and ghrelin, dissecting the putative effects of BMI on circulating ghrelin.

Our data are derived from a large cohort of NAFLD subjects, in whom insulin resistance, measured by the HOMA technique, was nearly the rule. The control group was carefully matched to account for potential confounders. Also, gender was considered, because a recent study demonstrated that ghrelin secretion is sexually dimorphic (18). Interestingly, our data showed that the gender effect is lost in NAFLD. Sex hormones may play an important role on ghrelin circulatory pattern (6, 19, 20), and estrogen therapy is a well-known cause of secondary NAFLD (21, 22). However, relative estrogen excess, as reported in advanced liver diseases, is unlikely in these subjects with normal liver function, and the result may simply stem from the low number of female patients causing type II statistical error.

To exclude differences related to BMI, our control population also included a few overweight or obese subjects with HOMA-R values in the range of insulin resistance as well. For this reason, ghrelin levels were analyzed in relation to quartiles of BMI and of insulin resistance, respectively. Similarly to data reported in other studies (23, 24), BMI played an important role on fasting ghrelin concentrations only in normal subjects.

By contrast, when data were analyzed in relation to quartiles of HOMA-R, the correlation between HOMA-R and ghrelin observed in controls was maintained in NAFLD, and HOMA-R was the sole factor predicting low ghrelin concentration in both groups at multivariate analysis.

The HOMA method for the measurement of insulin resistance has been extensively applied to epidemiological investigations. The figure of insulin resistance obtained with this method has a

relatively low reproducibility, which reflects day-to-day variability in fasting glucose and insulin, as well as analytical uncertainty. This is mainly the case for insulin levels, and a change of 1 μU/ml insulin may determine a change of up to 20% HOMA-R. Despite this, the method proved to correlate closely with quantitative, functional tests such as the glucose clamp technique (25, 26). In nondiabetic subjects, insulin concentrations account for the larger part of HOMA-R. This explains why insulin *per se* was also closely associated with ghrelin. However, in logistic regression, HOMA-R was mathematically preferred to insulin, suggesting that insulin resistance rather that insulin concentrations may regulate ghrelin secretion.

Low ghrelin levels are observed in several pathological conditions characterized by insulin resistance, such as moderate to severe obesity (2), polycystic ovary syndrome (6), acromegaly (27), and primary or secondary hypogonadisms (20), but the reason(s) for such relations are unclear. Ghrelin stimulates food intake (28), and reduced levels might be teleologically aimed at preventing the increase in body mass. This conclusion holds in controls, but not in NAFLD patients, in whom ghrelin is relatively independent of BMI. There is no consensus as to the exact relationship between ghrelin and insulin. Ghrelin was reported to stimulate (29) as well as to inhibit insulin secretion (30); in turn, hyperinsulinemia, either induced by a single bolus or during a clamp, produces conflicting effects on ghrelin concentrations. *In vitro* animal studies (31) and human studies during insulin infusion (5, 32) support the role of insulin as a secretagogue of ghrelin, but this effect was not confirmed in similar experiments in humans (33, 34). In our study, ghrelin was inversely related to insulin, but HOMA-R values were the most significant predictor of low ghrelin concentrations. Accordingly, ghrelin secretion might be under the control of insulin or, more likely, under the control of insulin resistance, via undefined circulating factors.

Insulin resistance was also tested by means of OGTT-derived indices. Although an overall correlation was observed in controls, this relation was lost in NAFLD. The figure of insulin resistance derived from ISI and SI is largely dependent on the dynamic response of glucose and insulin to the glucose load, which is not necessarily related to fasting values. This is mainly the case of NAFLD patients, who had a larger prevalence of glucose intolerance. The assessment of ghrelin response to oral glucose in NAFLD would be needed to compare the dynamic responses of ghrelin and insulin to glucose ingestion, and to assess the role of postload insulin resistance.

In search of other potential factors responsible for low ghrelin levels, we correlated hormonal levels with liver function parameters, with negative results. Only one study has been published so far on ghrelin levels in patients with liver disease. Tacke *et al.* (24) reported normal ghrelin levels in noncirrhotic patients and slightly elevated concentrations in cirrhosis. In their series of patients with advanced disease evaluated for liver transplantation, ghrelin was only related to the clinical severity of disease. This correlation, however, might also be spurious, and generated by the anorexia and decreased food intake of advanced disease. In our NAFLD cases, liver function was normal, and all subjects were on a controlled dietary regimen. Their BMI was similar to controls, and also excessive food intake cannot account for hormonal changes.

In conclusion, the study of NAFLD patients, in whom insulin resistance is relatively independent of obesity, strongly supports a primary role of insulin resistance *per se* on fasting ghrelin

concentrations. This confirms the importance of decreased insulin sensitivity on the multiple metabolic abnormalities of patients with NAFLD.

Footnotes

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Abbreviations: ALT, Alanine transaminase; BMI, body mass index; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; ISI, insulin sensitivity index; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OGTT, oral glucose tolerance test; OR, odds ratio; SI, sensitivity index; WHR, waist-to-hip ratio.

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1. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR** 2001 Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992–5995[Abstract/Free Full Text]
2. **Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML** 2001 Circulating ghrelin levels are decreased in human obesity. Diabetes 50:707–709[Abstract/Free Full Text]
3. **Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, Jorgensen JO** 2002 Weight loss increases circulating levels of ghrelin in human obesity. Clin Endocrinol (Oxf) 56:203–206[CrossRef][Medline]
4. **Tolle V, Kadem M, Bluet-Pajot MT, Frere D, Foulon C, Bossu C, Dardennes R, Mounier C, Zizzari P, Lang F, Epelbaum J, Estour B** 2003 Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. J Clin Endocrinol Metab 88:109–116[Abstract/Free Full Text]
5. **Lucidi P, Murdolo G, Di Loreto C, De Cicco A, Parlanti N, Fanelli C, Santeusanio F, Bolli GB, De Feo P** 2002 Ghrelin is not necessary for adequate hormonal counterregulation of insulin-induced hypoglycemia. Diabetes 51:2911–2914 [Abstract/Free Full Text]
6. **Pagotto U, Gambineri A, Vicennati V, Heiman ML, Tschop M, Pasquali R** 2002 Plasma ghrelin, obesity, and the polycystic ovary syndrome: correlation with insulin resistance and androgen levels. J Clin Endocrinol Metab 87:5625–5629 [Abstract/Free Full Text]
7. **Angulo P** 2002 Nonalcoholic fatty liver disease. N Engl J Med 346:1221–1231 [Free Full Text]
8. **Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M** 2003 Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 37:917–923[CrossRef][Medline]
9. **Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N** 1999 Association of nonalcoholic fatty liver disease with

insulin resistance. Am J Med 107:450–455[CrossRef][Medline]

10. **Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N** 2001 Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 50:1844–1850[Abstract/Free Full Text]
11. **Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR** 1999 Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474[CrossRef][Medline]
12. **Alberti KG, Zimmet PZ** 1998 Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med 15:539–553[CrossRef][Medline]
13. **World Health Organization** 2000 Preventing and managing the global epidemic: report of a WHO consultation. Geneva: World Health Organization, 2000
14. **Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschop M** 2001 Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur J Endocrinol 145:669–673[Abstract]
15. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and ß-cell function from plasma fasting glucose and insulin concentrations in man. Diabetologia 28:412–419[CrossRef][Medline]
16. **Matsuda M, DeFronzo RA** 1999 Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22:1462–1470[Abstract]
17. **Cederholm J, Wibell L** 1990 Insulin release and peripheral sensitivity at the oral glucose tolerance test. Diabetes Res Clin Pract 10:167–175[CrossRef][Medline]
18. **Barkan AL, Dimaraki EV, Jessup SK, Symons KV, Ermolenko M, Jaffe CA** 2003 Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. J Clin Endocrinol Metab 88:2180–2184 [Abstract/Free Full Text]
19. **Gambineri A, Pagotto U, Tschop M, Vicennati V, Manicardi E, Carcello A, Cacciari M, De Iasio R, Pasquali R** 2003 Anti-androgen increases circulating ghrelin levels in obese women with polycystic ovary syndrome. J Endocrinol Invest 26:629–634[Medline]
20. **Pagotto U, Gambineri A, Pelusi C, Genghini S, Cacciari M, Otto B, Castaneda T, Tschop M, Pasquali R** 2003 Testosterone replacement therapy restores normal ghrelin in hypogonadal men. J Clin Endocrinol Metab 88:4139–4143[Abstract/Free Full Text]
21. **Hamada N, Ogawa Y, Saibara T, Murata Y, Kariya S, Nishioka A, Terashima M, Inomata T, Yoshida S** 2000 Toremifene-induced fatty liver and NASH in breast cancer patients with breast-conservation treatment. Int J Oncol 17:1119–1123[Medline]
22. **Nemoto Y, Saibara T, Ogawa Y, Zhang T, Xu N, Ono M, Akisawa N, Iwasaki S, Maeda T, Onishi S** 2002 Tamoxifen-induced nonalcoholic steatohepatitis in breast cancer patients treated with adjuvant tamoxifen. Intern Med 41:345–350[Medline]
23. **Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, Purnell JQ** 2003 Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 88:174–178[Abstract/Free Full Text]
24. **Tacke F, Brabant G, Kruck E, Horn R, Schoffski P, Hecker H, Manns MP, Trautwein C** 2003 Ghrelin in chronic liver disease. J Hepatol 38:447–454[CrossRef] [Medline]
25. **Phillips DIW, Clark PM, Hales CN, Osmond C** 1994 Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurement of insulin resistance and insulin secretion. Diabet Med 11:286–292[Medline]
26. **Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M** 2000 Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23:57–63[Abstract]

27. **Cappiello V, Ronchi C, Morpurgo PS, Epaminonda P, Arosio M, Beck-Peccoz P, Spada A** 2002 Circulating ghrelin levels in basal conditions and during glucose tolerance test in acromegalic patients. Eur J Endocrinol 147:189–194[Abstract]
28. **Pinkney J, Williams G** 2002 Ghrelin gets hungry. Lancet 359:1360–1361[CrossRef][Medline]
29. **Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S** 2002 Ghrelin is present in pancreatic α-cells of humans and rats and stimulates insulin secretion. Diabetes 51:124–129[Abstract/Free Full Text]
30. **Egido EM, Rodriguez-Gallardo J, Silvestre RA, Marco J** 2002 Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. Eur J Endocrinol 146:241–244[Abstract]
31. **Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, Kangawa K, Matsukura S** 2001 Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. Biochem Biophys Res Commun 281:1220–1225[CrossRef][Medline]
32. **Saad MF, Bernaba B, Hwu CM, Jinagouda S, Fahmi S, Kogosov E, Boyadjian R** 2002 Insulin regulates plasma ghrelin concentration. J Clin Endocrinol Metab 87:3997–4000[Abstract/Free Full Text]
33. **Caixas A, Bashore C, Nash W, Pi-Sunyer F, Laferrere B** 2002 Insulin, unlike food intake, does not suppress ghrelin in human subjects. J Clin Endocrinol Metab 87:1902[Abstract/Free Full Text]
34. **Schaller G, Schmidt A, Pleiner J, Woloszczuk W, Wolzt M, Luger A** 2003 Plasma ghrelin concentrations are not regulated by glucose or insulin: a double-blind, placebo-controlled crossover clamp study. Diabetes 52:16–20[Abstract/Free Full Text]

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